100P Inverse Biomarker Exploring Technology (IBMET) for unveiling novel targets on TNBC: Mathematical identification of unknown targets based on structural features of epitopes specific for cancer cells

A. Imura[1], R. Maeda[1], H. Yamazaki[1], H. Yamazaki[2], T. Inoue[3], K. Hamamura[4], Y. Yoshida[4], N. Matsunaga[4], S. Ohdo[4], K. Araki[5], H. Tsukada[6], S. Aakashi-Tanaka[6]

[1]Cognano, Inc., Kyoto, Japan; [2]Biorhodes, Inc., Kyoto, Japan; [3]Faculty of Pharmacy, Graduate School of Pharmaceutical Sciences/Faculty of Pharmacy, Osaka University, Suita, Japan; [4]Faculty of Pharmacy, Kyusyu University, Fukuoka, Japan; [5]Research of Chemical Material, National of Institute of Advanced Industrial Science and Technology (AIST), Tokyo, Japan; [6]Breast Cancer Surgery, Tokyo Women's Medical University, Tokyo, Japan

Background: Currently, not only TROP2, EGFR, and VEGF—which are already approved—but also MUC1, Mesothelin, CEACAM6, GPNMB, CXCR4, and Claudin18.2 have been proposed as drug targets for HER2-negative breast cancers. These molecules are significantly overexpressed in cancer cells, enabling differentiation from normal cells and making them attractive candidates for drug development strategies such as antibody-drug conjugates (ADCs), T cell bispecific engagers (TCBs), and CAR-T therapies. If more specific target molecules could be identified, even more effective therapeutics might be developed. COGNANO, Inc. and collaborators aimed to discover novel target specific for unmet cancers.

Methods: We conducted long-term immunization of alpacas, which produce single-domain antibodies, using more than 10 types of human TNBC cell lines. Over time, we constructed a large library of VHH antibodies that recognize cell surface proteins. Leveraging this library, we developed an algorithm to cluster millions of antibody amino acid sequences *in silico* and identify characteristic clusters unique to each TNBC cell line. The Inverse Biomarker Exploring Technology (IBMET) is a bio-AI fusion platform that converts biologically generated single-domain antibodies into a digital library, enabling the discovery of novel biomarker molecules by using epitope-correlated features as indicators.

We validated antibodies recognizing novel markers in patient tissues and identified several biomarkers, including the most distinctive: VHH89T antigen.

Results: We formulated VHH89T into an ADC conjugated with the payload SN-38 and tested its cytotoxic activity in cultured cells, achieving highly favorable results. Additionally, its efficacy was confirmed in a mouse xenograft model.

Conclusions: The novelty and advantages of IBMET® include: Based on structural abnormalities, immediate drug formulation upon novel target discovery, as antibodies are already available. Parallel development of companion diagnostics, enabling rapid and comprehensive drug development. Here we propose a new Tech-Bio methodology suitable for immuno-oncotherapy.

Legal entity responsible for the study: The KYODOKEN Institutional Animal Care and Use Committee approved the protocols for these studies (approval number 20200312).

Funding: Kyoto Industrial Support Organization 21 The Ministry of Economy, Trade and Industry (METI), The Japan External Trade Organization (JETRO), Kyoto Prefecture Kyoto City.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.esmoop.2025.104261

101P Survival analysis and clinicopathological correlation with molecular classification in BCG-nonresponsive Ta-T1 bladder cancer

M.K. Singh, V. Singh, A. Kumar

Department of Urology, KGMU - King George's Medical University, Lucknow, India

Background: The heterogeneity of bladder cancer is defined by molecular subtyping, which aims to provide better risk stratification. This study investigated the association between survival analysis of non-muscle invasive bladder cancer (NMIBC) patients' molecular subtypes and the efficacy of intravesical Bacillus Calmette-Guérin therapy (BCG).

Methods: A total of 107 BCG non-responsive patients were enrolled with stage Ta high grade and/or T1 low and high grade. The patients were classified in luminal subtype marked by GATA3, CK20, and Uroplakin II and basal subtype; CK5/6 and CD44.The univariate and multivariate survival analyses were used to identify the association with clinicopathological parameters and molecular subtypes.

Results: In NMIBC, the relative expression of luminal markers defines subtype class 2 and basal markers define subtype class 3, whereas class 1 showed no relative expression of basal and luminal markers. The molecular subtype is an independent risk factor for recurrence-free survival (RFS) ($P = 0.041$) and progression-free survival (PFS) ($P = 0.044$) with high-grade and tumor-stage T1 in NMIBC. The molecular class shows a significant difference in the RFS ($P = 0.036$) and PFS($P = 0.019$) of the BCG-non-responsive patients.

Conclusions: The significance of molecular subtyping in predicting the efficacy of intravesical BCG therapy in NMIBC classes 2 and 3 showed independent correlations with RFS, whereas class 3 had PFS. These findings highlight the potential of immune histochemistry-based classification to aid risk stratification and support comprehensive pathological evaluation to improve the diagnosis and management of basal-like bladder cancer.

Legal entity responsible for the study: The authors.

Funding: Indian Council of Medical Research.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.esmoop.2025.104262

102P The predictive potential value of collagen type IX alpha 1 chain in colorectal cancer: Integrative analysis using RNA/DNA sequencing and pan-cancer studies

M. Maftouh[1], A. Avan[2]

[1]Medical Oncology, Amsterdam UMC - Vrije University Medical Centre (VUmc), Amsterdam, Netherlands; [2]Biomedical Sciences Dept., Queensland University of Technology, Brisbane, Australia

Background: Colorectal cancer (CRC) is the second leading cause of cancer-related deaths. This study aimed to identify new prognostic and therapeutic targets with particular focus on the extracellular matrix pathway.

Methods: This study aimed to provide a comprehensive analysis of the Collagen type IX alpha 1 chain (COL9A1) from a pan-cancer perspective employing multiomics data followed by validation in an additional cohort of CRC. The expression profile and function of COL9A1across different tumors were investigated to investigate the expression profile, genomic alterations, survival analysis, protein-protein interaction, correlation with immune cell subtypes, tumor immune microenvironment and enrichment analysis. Among the high top-score genes and dysregulated pathways associated with CRC, COL9A1 was detected and further validated in 120 CRC patients. Ccollagen family members were identified as core upregulated genes through protein-protein interaction network analysis followed by validation of expression and mutation analysis using RT-PCR and whole exome sequencing in CRC patients.

Results: Collagen family members were identified as core upregulated genes through PPI network analysis. The pan-cancer analysis demonstrated the consistent upregulation of COL9A1 across various cancers, including CRC. Moreover, the study explored the correlations between COL9A1 and immune infiltration to evaluate its potential as a therapeutic target and a guide for clinical treatment. The findings suggest that increased COL9A1 expression is associated with poorer overall and disease-free survival outcomes. The levels of COL9A1 promoter methylation were decreased.Functional enrichment analysis highlighted the critical role of COL9A1 in essential pathways, further underscoring its prognostic significance in CRC.

Conclusions: This study illustrated the comprehensive evidence supporting the importance of COL9A1 in the development and prognosis of CRC.

Legal entity responsible for the study: The authors.

Funding: Has not received any funding.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.esmoop.2025.104263

103P Molecular and immunohistochemical characterization of radiation-induced breast angiosarcoma

D. Erhan Keskin[1], M.E. El-Asrag[2], A. Shaaban[3], V. Heath[1], R. Bicknell[1], A.D. Beggs[4]

[1]Institute of Cardiovascular Sciences, The University of Birmingham, Medical School, Birmingham, United Kingdom; [2]College of Health and Life Sciences, Aston University, Birmingham, United Kingdom; [3]Histopathology, Queen Elizabeth Hospital - University Hospitals Birmingham NHS Foundation Trust, Birmingham, United Kingdom; [4]Institute of Cancer & Genomic Science, The University of Birmingham - Medical School, Birmingham, United Kingdom

Background: Angiosarcomas are rare yet highly aggressive malignant tumours originating from vascular endothelial cells. Treatment options are limited, and early diagnosis is critical for patient management. They can be classified into primary and radiation-induced (secondary) angiosarcomas. Secondary angiosarcomas have not yet been fully elucidated in terms of pathogenesis, clinical behaviour, early diagnostic biomarkers, genetic abnormalities and treatment targets. Identification of new biomarkers and therapeutic targets is important for improving the diagnosis and treatment of secondary breast angiosarcomas. This study is focused on secondary breast angiosarcoma arising as a consequence of radiotherapy following breast cancer treatment. These typically occur 5 to 10 years after radiation therapy. The objective of this study was to elucidate the immunohistochemical (IHC) profile of secondary angiosarcoma and determine the gene expression pattern of secondary angiosarcoma by performing RNA-seq analysis.

Methods: CD34, CD31, FLI-1 and ERG IHC stains were performed on 8 paraffin-embedded breast tissue sections diagnosed as secondary angiosarcoma. A total of 19 fresh frozen tissues were used for the RNAseq analysis of which 13 samples were angiosarcoma tumour tissue, while 6 samples were healthy tissue.

Results: CD31, CD34, and FLI1 were consistently highly expressed across four of the eight tumour samples, whereas ERG positivity was detected in only one of these four samples. Additionally, one tumour sample exhibited positivity solely for CD34 and FLI1, indicating variability in the expression of endothelial markers across cases. In the RNAseq analyses, 644 differentially expressed genes (DEGs) were identified, with 72 genes upregulated and 572 downregulated in secondary breast angiosarcoma. Gene Ontology (GO) enrichment analysis revealed that these DEGs were predominantly associated with biological processes like metabolism, energy pathways, and protein metabolism.

Conclusions: The findings presented highlight the heterogeneity in the expression of endothelial markers and transcriptomic profiles in secondary breast angiosarcoma, contributing to our understanding of this rare and aggressive malignancy.

Legal entity responsible for the study: The authors.

Funding: Ministry of National Education of Turkey.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.esmoop.2025.104264

104P Blood-derived immunomodulatory long non-coding RNAs signature in young Egyptian breast cancer patients: MIAT, HOTAIR, MALAT-1 and H19

S. Hany[1], A. Mokhtar[1], T. Manie[2], E. Khallaf[3], R.A. Youness[1]

[1]Molecular Biology and Biochemistry, GIU - German International University, Cairo, Egypt; [2]Breast Surgery Department, Cairo University - Faculty of Medicine, Cairo, Egypt; [3]General Surgery Department, Cairo University, Giza, Egypt

Background: The bona fide challenge that is recently evading the middle east countries and Egypt in particular is the undeniable alarming escalating incidence of BC among young females (<40 years old). Generally, the impression of BC patients in the middle east is that they present the disease at an earlier age and at a more advanced stage when compared to western countries. Concerning the current statistics in Egypt, the average age of Egyptian BC patients was found to be 10 years younger than American BC patients. Young Egyptian BC patients are 45x more likely to be diagnosed at stage III and 16x more likely to be diagnosed at stage IVcompared to old patients. Early diagnosis is the keyword for resolving such endemic crisis. Cell-free circulating nucleic acids such as Long non-coding RNAs (lncRNAs) in the blood have recently been casted as a new class of promising cancer diagnostic biomarkers. The aim of this study is to identify a blood derived lncRNA-signature for early diagnosis of young Egyptian BC patients.

Methods: A cohort of BC patients (n=55) were recruited. Solid tumor and its normal conuterpart were resected. All tumors (100%) were invasive ductal carcinoma. Median age was 35 years old (range 22-73). Lymph node involvment was reported in 72.7%. High Ki67 was observed in 83.6%. Stage 3 was reported in 54.5 % of patients. Tumor size >5 cm was recorded in 65.45%. Liquid biopsies were collected from the same patients. Age-matched healthy controls were recruited (n=40). RNA was extracted, reverse transcribed andquantifiedusing q-RT-PCR.

Results: An elevated expression pattern of lncRNAs: MIAT, HOTAIR, MALAT-1 and H19 in young BC patients tissues and plasma levels compared to the older group were observed. Upon patients' stratification, MIAT, HOTAIR and H19 expression levels were found to be associated with a high tumor grade, positive lymphnode metastasis, and high ki-67. However, MALAT-1 was highly elevated in more aggressive BC subtypes such as HER-2 and TNBC compared to luminal BC subtypes.

Conclusions: This study identify a blood derived lncRNA-signature for early diagnosis of young BC patients to be used as stable and non-invasive biomarker for such hard to diagnose and treat group of patients.

Legal entity responsible for the study: The authors.

Funding: L'Oreal UNESCO for Women in Science.

Disclosure: All authors have declared no conflicts of interest.

https://doi.org/10.1016/j.esmoop.2025.104265

105P Paired liquid biopsies analysis reveals resistance mechanisms and prognostic factors in patients treated with KRASG12C inhibitors

K. Beshiri[1], D. Vasseur[2], J.E. Rodriguez[1], C. Smolenschi[1], F. Facchinetti[3], A. Italiano[1], C. Nicotra[1], M. Ngo Camus[1], A. Gazzah[1], C. Baldini[1], L. Friboulet[2], Y. Loriot[1], F. Barlesi[3], C.P. Massard[1], A. Hollebecque[1]

[1]DITEP Departement, Institut Gustave Roussy, Villejuif, France; [2]Molecular Biology Department, Gustave Roussy - Cancer Campus, Villejuif, France; [3]Medical Oncology Departement, Institut Gustave Roussy, Villejuif, France

Background: KRASG12C inhibitors (G12Ci) represent an emerging class of therapeutics. We explored resistance mechanisms and prognostic factors using liquid biopsy (LB) analyses.

Methods: Retrospective analysis of LBs was conducted for patients (pts) treated with G12Ci from 2022 to 2024 under the STING trial (NCT04932525). Paired LBs (baseline and progressive disease [PD]) were evaluated using the FoundationOne Liquid CDx assay. Survival outcomes were analyzed using the "survival" and "survminer" R packages (v3.3-1 and 0.4.9).

Results: A total of 18 pts were included: NSCLC (n=9), colon cancer (n=8) and pancreatic cancer (n=1). All pts had a confirmed *KRASG12C* mutation in tissue samples. Overall, 21 pairs LBs were available: 13 included co-treatments (e.g., EGFR inhibitors [n=5], immunotherapy [n=4], small molecules [n=3]). At baseline, *KRASG12C* was detected in 18/21 pairs (85.7%), and in 15/21 at PD (71.4%). Three pairs showed no KRASG12C detection. Overall response rate was 42.8% (9/21). At PD, resistance-associated genomic alterations were observed in 28.6% (6/21), with a multiple mechanisms in four pts and unique in two. All six developed at least one on-target alteration affecting KRAS: two KRAS amplification, one KRAS R68S mutation, one *KRAS G12V* , one KRAS mutations at *G12A/D/F/S/Y, G13D, Q61H, R68S*, and *Y96D*, and one KRAS *G12A/F* and *R68S*. 3 *EGFR* mutations, a *MET amplification*, a E*TV6 fusion*, primarily associated with anti-EGFR resistance, and a *CDKN2A* mutation, were identified. Univariate analysis revealed that the number of metastatic sites at baseline (HR: 2.00, 95% CI: 1.30-3.00, p = 0.0013) was associated with worse PFS, as well as higher baseline VAF (HR: 3.40, 95% CI: 1.30-8.90, p = 0.015). These data were confirmed in multivariate analysis, where a VAF higher than the median (2.5%) remained a significant predictor of worse PFS (HR: 2.79, 95% CI: 1.01-7.75, p = 0.0049), as did the number of metastatic sites (HR: 1.84, 95% CI: 1.09-3.11, p = 0.022). Correlation between Tumor Fraction (cut-off 10%) and PFS was no statistically significant (p=0.081).

Conclusions: Paired LBs identified resistance mechanisms in 28.6% of cases with higher VAF at baseline and a greater number of metastatic sites associated with worse PFS.

Legal entity responsible for the study: The authors.

Funding: Has not received any funding.

Disclosure: D. Vasseur: Financial Interests, Personal, Invited Speaker: Roche, AstraZeneca. C. Smolenschi: Financial Interests, Institutional, Invited Speaker: Roche; Financial Interests, Personal, Invited Speaker: Servier; Financial Interests, Institutional, Other, Pi: BMS; Financial Interests, Personal, Other, Funding: Merck. A. Italiano: Other, Institutional, Other: Merck, Lilly, AstraZeneca, BMS, MSD, Bayer. C. Baldini: Financial Interests, Institutional, Advisory Board: BMS; Financial Interests, Institutional, Expert Testimony: MSD; Financial Interests, Personal, Invited Speaker: AstraZeneca; Financial Interests, Personal, Advisory Board: Janssen; Financial Interests, Institutional, Research Grant: BMS; Financial Interests, Institutional, Invited Speaker: iTeos, AZ, Janssen, Amgen, Bicycle Therapeutics, MSD, Seattle Genetics, Taiho, Pyramid Bioscience, Tango, Roche Genentech; Non-Financial Interests, Personal, Member: ASCO, SIOG, SOFOG, AACR, ESMO. L. Friboulet: Financial Interests, Institutional, Other: Nuvalent, Sanofi, Debiopharm, Incyte, Relay Therapeutics. Y. Loriot: Financial Interests, Personal, Advisory Board: Merck Kga, Pfizer, Gilead, Seattle Genetics, Taiho; Financial Interests, Personal, Other, lectures, advisory boards: MSD, AstraZeneca, Astellas, Janssen; Financial Interests, Personal, Other, lectures, advisory boards: Roche, BMS; Financial Interests, Institutional, Research Grant: Janssen, Sanofi, MSD, Roche, Celsius; Financial Interests, Institutional, Invited Speaker: Janssen, Pfizer, Janssen, MSD, Janssen, Exelixis, AstraZeneca, Pfizer, Merck Kga, BMS, Astellas, Gilead, Incyte; Financial Interests, Personal, Invited Speaker: MSD, Astellas, Gilead/Immunomedics, Basilea, Taiho; Non-Financial Interests, Personal, Member: ESMO, ASCO, AACR; Non-Financial Interests, Personal, Other, scientific committee: ARC. F. Barlesi: Financial Interests, Institutional, Advisory Board: AstraZeneca, Bayer, Bristol Myers Squibb, Boehringer Ingelheim, Eli Lilly Oncology, F. Hoffmann−La Roche Ltd., Novartis, Merck, Mirati, MSD, Pierre Fabre, Pfizer, Sanofi Aventis, Seattle Genetics, Takeda, AbbVie, ACEA, Amgen, Eisai, Ignyta, Summit Therapeutics; Non-Financial Interests, Personal, Principal Investigator: AstraZeneca, BMS, Merck, Pierre Fabre, F. Hoffmann-La Roche Ltd., Innate Pharma, Mirati. C.P. Massard: Other, Personal, Other, Christophe Massard: Consultant/Advisory fees from Amgen, Astellas, AstraZeneca, Bayer Ipsen, Janssen, MSD, Novartis, Pfizer, Roche, Sanofi, Netcancer, PegascyPrincipal/sub-Investigator of Clinical Trials for AbbVie, Aduro, Agios, Amgen, Argen-x, Astex, AstraZeneca, Aveo Pharmaceuticals, Bayer, Beigene, Blueprint, BMS, Boeringer Ingelheim, Celgene, Chugai, Clovis, Daiichi Sankyo, Debiopharm, Eisai, Eos, Exelixis, Forma, Gamamabs, Genentech, Gortec, GSK, H3 Biomedecine, Incyte, Innate Pharma, Janssen, Kura Oncology, Kyowa, Lilly, Loxo, Lysarc, Lytix Biopharma, MedImmune, Menarini, Merus, MSD, Nanobiotix, Nektar Therapeutics, Novartis, Octimet, Oncoethix, Oncopeptides AB, Orion, Pfizer, PharmaMar, Pierre Fabre, Roche, Sanofi, Servier, Sierra Oncology, Taiho, Takeda, Tesaro, Xencor. A. Hollebecque: Financial Interests, Personal, Invited Speaker: Servier, Incyte, Eisai, BMS; Financial Interests, Personal, Advisory Board: Basilea, Taiho, Relay Theraeutics, QED Therapeutics, Debiopharm, MSD, Boehringer Ingelheim; Financial Interests, Institutional, Funding: Incyte; Financial Interests, Institutional, Research Grant: AstraZeneca; Non-Financial Interests, Personal, Principal Investigator, M19-345; M21-404: AbbVie; Non-Financial Interests, Personal, Principal Investigator, CO42216; WP42627; CO40939; GO44479; GO42216: Roche; Non-Financial Interests, Personal, Principal Investigator, MCLA-158; MCLA-128: Merus; Non-Financial Interests, Personal, Principal Investigator, SGNB6A: Seatle Genetics; Non-Financial Interests, Personal, Principal Investigator, TAS-120-202: Taiho; Non-Financial Interests, Personal, Principal Investigator, Krystal-10: Mirati; Non-Financial Interests, Personal, Principal Investigator, ADP-0033: Adaptimmune; Non-Financial Interests, Personal, Principal Investigator, ACT16902: Sanofi; Non-Financial Interests, Personal, Principal Investigator, C4201002; SGNB6A: Pfizer; Non-Financial Interests, Personal, Principal Investigator, RLY-4008: Relay Therapeutics; Non-Financial Interests, Personal, Principal Investigator, CC-90011: Celgene/BMS; Non-Financial Interests, Personal, Principal Investigator, Loxo-IDH; Loxo-RAS: Loxo/Lilly; Non-Financial Interests, Personal, Principal Investigator: AstraZeneca; Non-Financial Interests, Personal, Principal Investigator, SN-201 study: Sotio; Non-Financial Interests, Personal, Principal Investigator, Tropics-03: Gilead; Non-Financial Interests, Personal, Principal Investigator, BI1403: Boehringer Ingelheim; Non-Financial Interests, Personal, Principal Investigator, CA120-1001: BMS. All other authors have declared no conflicts of interest.

https://doi.org/10.1016/j.esmoop.2025.104266